

TO ALL TO WHOM THESE PRESENTS SHALL COME;

UNITED STATES DEPARTMENT OF COMMERCE United States Patent and Trademark Office

June 4, 1999

JUNE

1999 S

PCT

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/084,731

FILING DATE: May 8, 1998

PRIORITY DOCUMENT

COMPLIANCE WITH RULE 17.1(a) OR (b)



By Authority of the

COMMISSIONER OF PATENTS AND TRADEMARKS

H. L. JACKSON

Certifying Officer

Best Available Copy

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

05/08/98 05/08/98
6679

			Docket Number	0698.072/1	Prov.		a plus sig e this box		+
_			INVENTOR	(s)/APPLICANT(S)				
Last Na	Last Name First Name Middle Initial Residence (City and Either State or Foreign Country)								
ROZHET	HETSKY KARINA Haifa, ISRAEL								
		TITLE	OF THE INVE	NTION (280 chara	cters max)			_
CAR	BOXYI	LIC CATION	ITES AND A	METHOD FO	R THEI	R MA	ANUFAC	CTURE	
-			900 17 TH S	STREET, N.W.					
STATE	WASHI	NGTON, D.C.	ZIP CODE	20006	COUNT	COUNTRY USA			·
		ENCLOSE	D APPLICATION	ON PARTS (check	all that ap	ply)	· 		_
Specification Number of Pages Small Entity Statement Drawing(s) Number of Sheets Other (specify): Claim(s) Number of Pages									
		1	METHOD OF P	AYMENT (check of	ne)				
■ Th	A check or money order is enclosed to cover the Provisional filing fees. The Commissioner is hereby authorized to charge any additional filing fees Amount(s) \$75.00)		
	ı was mad	ie by an agency		ates Government of		contra	ct with an	agency of t	he

Date 5 / 8 / 98

(if appropriate)

REGISTRATION NO. 25,109

Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted SIGNATURE

TYPED or PRINTED NAME Herbert Cohen
WIGMAN, COHEN, LEITNER & MYERS, P.C.

The Farragut Building, 10th Floor 900 17th Street, N.W., Suite 1000

Washington, D.C. 20006

(202) 463-7700 - Phone

Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

15

20

Carboxylic cationites and a method for their manufacture

Abstract

The invention refers to preparation of synthetic polymerized resins for ion exchange, namely, carboxylic cationites. These cationites are suitable for using in medicine, microbiological industry and food industry as sorbents for preparative separation and purification of biologically active compounds by low pressure liquid chromatography (LPLC).

The directional polymerization conditions make it possible to prepare cationites with polymeric structures which can be used for separation of proteins and enzymes of high molecular mass (MM) from various microbiological raw materials and physiological liquids.

Field of the Invention

Carboxylic cationites for the separation and purification of biologically active substances are known and widely used for sorption and desorption of various organic substances like proteins and enzymes, and one of the advantages of these cationites lies in the fact that they do not cause denaturation of the proteins and enzymes in the course of separation. Unfortunately, the known-in-the-art carboxylic cationites are mostly defined by a macroporous structure which has a limited exchange capacity with respect to high MM proteins and therefore are less effective for separation of high MM proteins.

Also described in the art are chromatographic methods based on ion exchange chromatography and employing sorbent-materials based on various polysaccharides known under the trade names sephanose, sephadex and others. Unfortunately these sorbent materials have only limited sorption ability and are incapable of biologically separating active substances from complex mixtures in the presence of mineral salts. Therefore they are usuable merely after the stage of desalting. It should also be pointed out also that the chemical stability of these materials is low, and accordingly, their service life expressed as number of regeneration cycles is insufficient.

30

1027

There is a known-in-the-art process for the production of carboxylio cationites as disclosed in US patent No.4120831. This process involves the co-polymerization of methacrylic or scrytic acids with suitable crosslink agents, e.g., hexahydro-1,3,5-triacryloyltriazine (HTA), N,N'-methylendyscrylamide (MDAA), N,N'-ethylendymethacrulamide (EDMA), or N,N'-hexamsthylendymethacrylamide (HMDMA).

· 🎶

The co-polymerization is carried out in a water solution of acetic acid having a concentration of 5-50 weight %. After the short period of pre-polymerization the reaction mixture is dispersed within a dispersing medium which is liquid polyethylensiloxane or polymetylensiloxane. The resulting carboxylic cationites are obtained in a form of spherically-shaped granules. The carboxylic cationites prepared by the above described process do not possess sufficient sorbtion capacity required for the effective separation of proteins and enzymes. A possible reason for this is the surface tension of the reaction mixture created at the condition of co-polymerization carried out within a medium of liquid polysiloxane. Due to this tension the size of pores formed on the outside surface of granules is decreased.

The further disadvantage of the known method is associated with the migration of components of the reaction mixture (co-monomers and acetic acid) into the dispersion medium. The migration occurs during the co-polymerization and makes it very difficult to prepare the final product with reproducible structural properties.

Summary of the Invention

The main object of the present invention is to produce carboxylic cationites and a method for their manufacturing which which will sufficiently reduce or overcome - the above mentioned disadvantages of known-in-the-prior art carboxylic cationites.

In particular, the main object of the invention is to produce for a new and improved carboxylic cationite which has a high sorbtion capacity with respect to high MM biologically active substances, e.g. proteins and enzymes.

A further object of the invention is to produce a carboxylic cationite which can be easily and conveniently manufactured and which is defined by a structure with reproducible properties.

Still a further object of the invention is to produce a new and improved method for manufacturing of carboxylic cationites which does not require the use of silicone

io io io

人名英格兰人姓氏

京の記を選を選びている

20

5

10

15

5

10

15

20

25

liquids as a dispersion medium and in which the particulated cationite is obtained by dispersion of the reaction mixture within an aqueous solution of a mineral salt.

The above and other objects and advantages of the invention can be achieved by the following combination of its essential features which are summarized briefly. The reaction mixture of co-monomers is dissolved in a solvent, which can be chosen from the group comprising water, acidic acid having a concentration of 5-15 weight % or 60-100 weigh percent, organic water-soluble solvents or organic water-insoluble solvents.

Before dispersing of the reaction mixture within a dispersing medium the pre-polymerization process is carried out at conditions enabling the obtaining of a high viscous polymeric structure. The resulting particulate product consists of granules having irregular form with the mean size of 1-500 microns and defined by a porous structure. The size of pores varies between 2 to 500Å. The resulting product has improved sorbtion capacity with respect to high MM proteins and enzymes and is suitable for isolation and purification of a wide range of proteins.

Detailed Description of Specific Embodiments

The ability of a carboxylic cationite to selectively and efficiently absorb proteins and enzymes is associated with the chemical nature of the employed co-monomers, the topology of the polymeric matrix.

In order to increase the size of pores of the sorbent without substantially reducing the volume concentration of the functional groups in the polymeric matrix, the co-polymerization stage is carried out in a water solution of the acetic soid or in other water-soluble solvents such as dimethilformamide monobutyl glycolate, polyethylenglycol or water-insoluble solvents, such as butyl alcohol, octyl alcohol, decyl alcohol. Each of these solvents can be used alone or in any combination thereof.

Monovinyl monomers and a crosslink agent are employed as co-monomers. The suitable monovinyl monomers comprise methacrylic acid, acrylic acid, methacryloylcaproic acid, methacryloylaminobenzoic acid, methacryloylglycine, etc. These monomers also can be used alone or in any combination thereof. The crosslink monomers suitable for the present invention comprise polyvinylmonomers such as EDMA, HMDMA. MDAA. triaallylisocyanurate (TAIC),

p-phenylendimethacrylamid(p-PHDMA) benzidimedimethacrylamide (BDMA) etc. In practice it is advantageous to use HTA, EDMA and TAIC.

In order to initiate the polymerization process a free radical initiator such as a suitable redox system is used; e.g., ammonium persulfate-sodium methabisulfite, ammonium persulfate, 1,1'- azobis(cyclohexanecarbonitrile), etc.

The co-polymerization step is carried out within a solvent, which is chosen from the group comprising water, a solution of acidic acid having concentration of 5-15 weight % or 60-100 weight %, organic water-soluble solvents or organic water-insoluble solvents. The amount of co-monomers in the reaction solution should be 10-30 weight %.

The dispersion step is carried out within a dispersing medium, which is an aqueous solution of inorganic salts.

By virtue of the present invention it is possible to obtain carboxylic cationites defined by reproducible properties and superior sorption performances in comparison to the cationites prepared by the known-in-the-art method. For example, the bovine serum albumin sorption output obtained with the new carboxylic cationites was 1090 mg/g whereas the sorbtion output achieved with the outionites prepared by dispersion in polysiloxane liquids was only 180 mg/g.

It should be pointed out that the known methods employing polysiloxane liquids require washing the resulting cationite by petroleum, ether, acetones or other organic solvent. These solvents are flammable liquids and therefore appropriate procautions should be taken so as to carry out the process safely on an industrial scale. It can be readily appreciated that the necessity in additional safety measures complicates the whole manufacturing process and renders it more expensive. Since the present invention employs a dispersing media which does not require washing in flammable solvents the proposed manufacturing method is simple, cheap and easily adaptable to an industrial scale.

In practice the carboxylic cationites of the present invention are manufactured by dissolution of the co-monomers, i.e., monovinyl monomers and a polyvinyl-containing cross-link agent, which are taken in the weight ratio from 8:1 to16:1. The co-polymerization is carried out in the presence of an initiator of radical polymerization. The solution of the reaction mixture prior is subjected to prepolymerization at a temperature of 20-25°C if the initiator is a redox system or by a

n j j 15

5

10

20

25

-30

temperature of 50-60 °C if the initiator nersulfite 1-1'-azobis(cyclohexanecarbonitril).

Then the reaction mixture is dispersed within the dispersion medium, which is 20 weight % aqueous solution of sodium sulfate.

The weight ratio of the reaction mixture to dispersion medium varies from 1:3 to 1:5 respectively. The obtained particulate cationite is separated from the dispersion medium and washed out by water, 0.5-1N aqueous solution of sodium hydroxide, water so as to obtain pH 8, 1 N aqueous solution of hydrochloric acid and again by water.

After washing, the particulate cationite is dried in open air and its structure parameters and performances are tested.

The loose weight of a particulate cationite can be referred to as an index of porosity. In the present invention the loose weight was determined according to the following procedure:

A sample fraction of air dried loose cationite with a particle size of about 100 microns was put in a cylinder and the volume of the sample was measured. Then its weight was measured. The loose density was calculated by dividing the weight of the loose sample to its volume. The loose weight of the cationite of the present invention lies in the range 0.2 -0.7 g/ml, preferably being 0.4 -0.7 g/ml.

The swelling factor of cationites in the H+ form in water, as well in a phosphate buffer was measured by dividing the specific volume in the swollen state by the volume of the dried sample. The specific volume in the swollen state was measured by dividing the volume of swollen sample by the weight of dry sample.

Exchange capacity with respect to a sodium ion was defined by using a 0.5 N solution of NaOH in the presence of 0.1 N solution of NaCl.

Size of pores was measured by scanning electronic microscope on samples prepared in accordance with the following procedure. The sample of a cationite is held within 50 weight % acidic acid for 5 hours. The sample is dehydrated gradually in ethanol. Very thin slices are cut and treated by lead citrate so as to improve contrast. Before carrying out the measurement the sample is fixed by the solution of osmium oxide and uranilacetate solution.

The new carboxylic cationites are obtained in particulate form with particle size 1-500 microns and are defined by good hydrodynamic properties. They have

10

15

20

5

10

15

20

25

insignificant swelling change (1.2-1.8) due to transformation from the H+ form into hydrogen-sodium form upon changing the pH value from 4 to 7, i.e. under the conditions of the sorption-desorption process. This property is in accordance with the requirement of reversible sorption of proteins and enzymes within the pH range from 4 to 7. The cationites are chemically stable in the pH range of 1-14 and enable many cycles of regeneration. The cationites are thermally stable at 125°C. The flow rate of a solution, containing biologically active substances, passed through the column filled with a carboxylic cationite of the present invention varies in the range 200- 300 ml/hrcm2, depending on the structure of the cationite and when the cationite is in the hydrogen form in the swollen state. The important advantage of the cationites of the present invention is their ability to absorb selectivity and fully reversibly the proteins and enzymes of various nature and molecular weight from sait solution. Therefore one of these sorbents may eliminate the necessity for desalination of the starting mixtures prior to the process of ion exchange separation. The new cationites have been shown as having high sorption ability with respect to various proteins. For example, the sorption output with respect to Hemoglobin (Hb) is 220 mg/ml, with respect to Bovin scrum albumin (BSA) is 180 mg/ml, with respect to γ - Globulin (γ - Gl) is 80.5 mg/ml, with respect to Human serum albumin (HAS) is 70 mg/ml, and with respect to Goat scrum albumin (GSA) is 80mg/ml of swollen sorbent. By using carboxylic estionites of the present invention it was possible to isolate and purify the immunoglobuline G1 (Ig G1) from raw mouse ascites fluid with 95.8% purity and about 100% yield (determined by HLISA analysis) thus avoiding additional purification stages. Sorbents of this invention are advantageous for use in low pressure liquid chromatography (LPLC) for industrial separation and purification of proteins and enzymes from naturally-occurring sources, as well as for preparative purposes for manipulation with proteins, enzymes and oligopeptides.

This invention will be further illustrated by the following examples, which by no means limit the scope of the invention

Example I

250 ml of 20 % sodium sulfate solution are poured into a 500 ml flask provided with a stirrer, an inlet pipe for argon and a dropping funnel, and argon is bubbled for 30 minutes. The sodium sulfate solution will be used as a dispersing medium.

10

15

20

25

At the same time in a current of argon charged into the dropping funnel are charged 10 ml of methacrylic acid, 1.22g of HTA and 45.5 ml of water. After dissolution of these co-monomers into the funnel an initiator is added, i.e., 0.0426 g of ammonium persulfate and 0.0143 g of sodium methabisulfite. The solution of reaction mixture is maintained in the funnel at a temperature of 22°C for 5-7 minutes. Then the resulting pre-polymer is poured into the dispersion medium under stirring with velocity 800-1000 rpm for 40 minutes Further, the flask temperature is increased to 60°C and the resulting suspension is stirred at this temperature for 30 minutes. Then the flask temperature is increased to 100°C and the suspension is maintained at this temperature under stirring for one hour. After cooling to room temperature the resulting cationite particles are separated from the dispersion medium and washed out with water, 1N solution of sodium hydroxide, water to pH 8, 1N solution of hydrochloric acid and with water. The yield of cationite in the air-dry state is 10.9g which is 96%; The predominant particle size is 100-200 µm. The exchange capacity with respect to a sodium ion is 10.3 mg-equiv/g of sorbent. The awelling factor of the cationite in the H-form in water is 4.5. The swelling factor of the cationite in the H- form in a phosphate buffer (pH7) is 6.6. The specific volume in the swollen state is 6.5 ml/g. The corption capacity with respect to BSA (MM 66000) is 115g/ml, to Hb (MM 66000) is 110g/ml, to \gamma-Gib (180000) is 80.5 g/ml of swollen sorbent. The yield of proteins upon desorption is 97-100%.

Example 2

250 ml of 20% sodium sulfate solution are poured into a 500 ml flask provided with a stirrer, an inlet pipe for argon and a dropping funnel, and argon is bubbled for 30 minutes. The sodium sulfate solution will be used as a dispersing medium. At the same time in a current of argon into the funnel there are charged 10 ml of methacrylic acid, 1.22g of HTA and 45.5 ml of 70% acidic acid. After dissolution of the co-monomers into the funnel an initiator is added, i.e., 0.0569g of 1-1'azobis(cyclohexanecarbonitrile).

The solution of reaction mixture is maintained in the funnel at a temperature of 22°C for 5-7 minutes. Then the resulting pre-polymer is poured into the dispersion medium under stirring with velocity 800-1000 rpm for the 10 minutes. Further, the flask temperature is increased to 60°C and the resulting co-polymer suspension is

15

20

25

stirred at this temperature for 40 minutes. Then the flask temperature is increased to 100°C and the copolymer suspension is maintained at this temperature and understirring for one hour. After cooling to room temperature the cationite particles are separated from the dispersion medium phase and washed out with water, 1N solution of sodium hydroxide, water to pH 8, 1N solution of hydrochloric acid and with water. The yield of cationite in the air-dry state is 10.9g which is 95%. The predominant particle size is 100-200 microns. The exchange capacity with respect to a sodium ion is 10.23 mg-equiv/g of sorbent. The swelling factor of the cationite in the H-form in water is 3.3. The swelling factor of the cationite in the H- form in a phosphate buffer (pH7) is 6. Specific volume in the swollen state is 7ml/g. Sorption capacity with respect to BSA (MM 66000) is 80g/ml, to y-Gib (MM180000) is 75.4 g/ml of swollen sorben. The yield of proteins upon description is 97-100%.

Example 3

250 ml of 20% sodium sulfate solution are poured into a 500 ml flask provided with a stirrer, an inlet pipe for argon and a dropping funnel, and argon is bubbled for 30 minutes. This solution will be used as a dispersion medium.

At the same time in a current of argon into the funnel there are charged 10 ml of methacrylic acid, 1.22g of HTA and 45.5 ml of 5% acidic acid. After dissolution of the above co-monomers into the funnel an initiator is added, i.e., 0.0426g of ammonium persulfate and 0.0143 g of sodium methabisulfite. The solution of reaction mixture is maintained in the funnel at a temperature of 22°C for 5-7 minutes. Then the resulting pre-polymer is poured into the dispersion phase under stirring with a velocity of 800-1000 rpm. Thereafter co-polymerization of the resulting suspension is carried out by a temperature 22°C under the stirring for 40 minutes. Further, the flask temperature is increased to 60°C and the suspension is stirred at this temperature for 30 minutes. Then the flask temperature is increased to 100°C and the suspension is maintained at this temperature and under stirring for one hour. After cooling to room temperature cationite particles are separated from the dispersion phase and washed out with water, 1N solution of sodium hydroxide, water to pH 8, 1N solution of hydrochloric acid and with water. The yield of cationite in the air-dry state is 10.8g which is 94.5%; The predominant particle size is 100-200 microns. The exchange capacity with respect to sodium ion is 10.3 mg-equiv/g of sorbent. The swelling factor

の報信のある

Sec. 15.

of the cationite in the H-form in water is 4.1. The swelling factor of the cationite in the H-form in a phosphate buffer (pH7) is 5.7. Specific volume in the swellen state is 6 ml/g. Sorption capacity with respect to BSA (MM 66000) is 180g/ml, to Hb (MM 66000) is 220g/ml, to y-Gib (MM 180000) is 60 g/ml of swellen sorbent. The yield of proteins upon desorption is 97-100%.

Example 4

5

10

15

20

25

30

250 ml of 20% solution of sodium sulfate are poured into a 500 ml flask provided with a stirrer, an inlet pipe for argon and a dropping funnel, and argon is bubbled for 30 minutes. This solution will be used as a dispersing medium. At the same time in a current of argon into the funnel there are charged 10 ml of methacrylic acid, 1.22g of HTA and 45.5ml of a solvent, consisting of 44.5ml of water and 1ml of polyethylenglycol. After dissolution of the above co-monomers into the funnel an initiator is added, i.e., 0.0569 g of ammonium persulfate. The solution of reaction mixture is maintained in the funnel at a temperature of 50°C for 5-7 minutes. Then the resulting pre-polymer is poured into the dispersion phase under stirring with a velocity of 800-1000 rpm for 10 minutes. Further, the flask temperature is increased to 60 °C and the copolymer suspension is stirred at this temperature for 40 minutes. Then the flask temperature is increased to 100°C and the suspension is maintained at this temperature and under stirring for one hour. After cooling to a temperature of 25 °C the cationite particles are separated from the dispersion phase and washed out with water, 1N solution of sodium hydroxide, water to pH 8, 1N solution of hydrochloric acid and again with water. The yield of cationite in the air-dry state is 10.95g which is 96.3%; The predominant particle size is 100-200 microns. The exchange capacity with respect to sodium ion is 10.3 mg-equiv/g of sorbent. The swelling factor of the cationite in the H-form in water is 1.4. The swelling factor of the cationite in the IIform in a phosphate buffer (pH7) is 2. Specific volume in the swollen state is 4.3 ml/g. Sorption capacity with respect to BSA (MM- 66000) is 90g/ml, to 7-Gib (MM 180000) is 64 g/ml of swollen sorbent. The yield of proteins upon desorption is 97-100%.

Example 5

はおり

上の多数をです。

The state of the s

250 ml of 20% solution of sodium sulfate are poured into a 500 ml flask provided with a stirrer, an inlet pipe for argon and a dropping funnel, and argon is bubbled for 30 minutes. This solution will be used as a dispersing medium. At the same time in a current of argon into the funnel there are charged 10 ml of methacrylic acid, 1.22g of HTA and 45.5ml of a solvent, consisting of 44.5ml of 2-butyl alcohol. After dissolution of the above co-monomers into the funnel an initiator is added, i.e., 0.0569 g of 1,1'azobis(cyclohexanocarbonitril). The solution of reaction mixture is maintained in the funnel at a temperature of 60°C for 5-7 minutes. Then the resulting pre-polymer is poured into the dispersion phase under the stirring. Thereafter co-polymerization is carried out in suspension at 22 °C under stirring with a velocity of 800-1000 rpm for 10 minutes. Further, the flask temperature is increased to 60 °C and the copolymer suspension is stirred at this temperature for 40 minutes. Then the flask temperature is increased to 100°C and the suspension is maintained at this temperature and under stirring for one hour. After cooling to a temperature of 25 °C the cationite particles are separated from the dispersion phase and washed out with ethyl alcohol, water, 1N solution of sodium hydroxide, water to pH 8, 1N solution of hydrochloric acid and again with water. The yield of cationite in the air-dry state is 10.85g which is 95.4%; The predominant particle size is 100-200 microns. The exchange capacity with respect to sodium ion is 10.3 mg-equiv/g of sorbent. The swelling factor of the cationite in the H-form in water is 3 2. The swelling factor of the cationite in the H- form in a phosphate buffer (pH7) is 5.6. Specific volume in the swollen state is 6.5 ml/g. Sorption capacity with respect to BSA (MM- 66000) is 40g/ml, to y-Gib (MIM 180000) is 34 g/ml of swollen sorbent. The yield of proteins upon description is 97-100%.

25

20

5

10

The Sandard Control

		Attorney Docket 0698.072/1
00282 County St. Pati	entee: Karina Rozhetsky	
erial or Patent	No.: Filed	or respect Way 8, 1998
		OD FOR THEIR MANIFACTURE
· VERG	FIED STATEMENT (DECLARATION 37 C.Y.R. 1.9(f) and 1.27(c	c)) - 240TT Breinese Concess B) Cyvining Emait Sullia Blyine
hereby declare	that I om	
[M] en offi	n identified below:	s donoern empowered to act on Denail Of Che
ADDRESS OF COME	POLY	GRAN LTD.
ADDRESS OF C	XNCERN 99 HARIST	ADRUT AVE . P.O.B . 25321 1 3/250 /SRAFL
imall business of 1.9(d), for purp inited States On the affiliate the number of fiscal year of temporary basis are affiliates controls or has or has the power thereby declary	poncern as defined in 19 C poses of paying reduced fee pode, in that the number of es, does not exceed 500 per employees of the business the concern of the parse during each of the pay pe of each other when eit the power to control the or r to control both.	ied small business concern qualifies as a r.R. 121.3-19, and reproduced in 37 C.F.R. as under Section 41(a) and (b) of Title 35, ampleyess of the concern, including those recors. For purposes of this statement, (1) concern is the average over the previous one employed on a full-time, part-time or priods of the fiscal year, and (2) concerns ther, directly or indirectly, one concern other, or a third party or parties controls not or law have been conveyed to and remain
with the small entitled	Duelness concern identification (ARAOXYLIC (4710) MANUFACTURE	fied above with regard to the invention, N ITES AND A METHOD FOR THEM
described in [#] the sp [] applio		, 100000
[] patent	ald by the shows identific	od small husiness concern are not exclusive. I having rights to the invention is listed
If the rights heach individual below and no a tor, who could any concern wh	rights to the invention are not qualify as a small bu- nich would not qualify as- norofit organization under Separate verified states weream, concern or organi-	siness concern under 37 C.F.R. 1.9(d) of by a small business concern under 37 C.F.R
If the rights he sain individual below and no stor, who could any concern who 1.9(d) or a nor "NOTE:	rights to the invention are not qualify as a small but such would not qualify as approfit organization under Separate verified states person, concern or organizating to their status a	member concern under 37 C.F.R. 1.7(4) of D member and the concern under 37 C.F.R 37 C.F.R. 1.9(e). ments are required from each named zation having rights to the invention as small entities. (37 C.F.R. 1.27)
f the rights heach individual below and no stor, who could any concern who 1.9(d) or a nor *NOTE: NAME ADDRESS NAME ADDRESS	rights to the invention are not qualify as a small but such would not qualify as approfit organization under Separate verified states person, concern or organizating to their status a	n small business concern under 37 C.F.R. 1.9(a) on a small business concern under 37 C.F.R. 37 C.F.R. 37 C.F.R. 1.9(a). ments are required from each maned zation having rights to the invention as small entities. (37 C.F.R. 1.27) INESS CONCERN [] NONPROFIT ORGANIZATION

further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, inder Section 1001 of Title 18 of the United States Orde, and that such willful false statements may proportize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING	Karina	Rozhe	etsky		
TITLE OF PERSON OTHER THUN CHARER	Gener	al m	anka	CA	
Address of Person Signing	32 Pear	St . 14	21-0	15RA	4
1			- 0		
SIGNATURE Say		DAT	ue	6/1V98	
				/ ~ .	10158

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:
BLACK BORDERS
IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
☐ FADED TEXT OR DRAWING
☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
☐ SKEWED/SLANTED IMAGES
☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
☐ GRAY SCALE DOCUMENTS
☐ LINES OR MARKS ON ORIGINAL DOCUMENT
☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
OTHER:

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.